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VOL. 20, SEC. A. DECEMBER, 1942 NUMBER 12

NOTE ON THE ENERGY AND MOMENTUM CORRECTION FACTORS FOR FLOW IN CIRCULAR PIPES[1]

BY K. F. TUPPER[2]

Abstract

The energy and momentum correction factors are expressed as functions of two integrals depending on the velocity distribution. General relations for any stream cross-section are given for the sign and relative size of the integrals. Using the Karman–Prandtl velocity distribution laws for circular pipes the integrals are evaluated numerically and given as functions of the bulk Reynolds number, the pipe friction factor, and other useful quantities likely to be known in practice.

Definitions

The kinetic energy in the fluid which passes a given section of a stream per unit time is

$$E = \int_A \tfrac{1}{2}\rho v^2 u\,da,$$

and the component of the linear momentum normal to the plane of the section is

$$M = \int_A \rho u^2\,da,$$

where ρ = mass density of the fluid,

v = velocity at a point on the section,

u = normal component of the velocity,

A = the total area of the section and,

da = the differential area.

In most of the cases where the correctio ı factors can be applied, the stream will be of constant section and the velocity sufficiently close to the purely axial that it may be assumed that $u = v$, in which case

$$E = \int_A \tfrac{1}{2}\rho u^3\,da.$$

[1] *Manuscript received in original form July 25, 1942, and as revised, October 10, 1942. Contribution from the Division of Mechanical Engineering, National Research Laboratories, Ottawa, Canada. Issued as N.R.C. No. 1093.*

[2] *Physicist.*

Energy and momentum are usually expressed in terms of the mean velocity $\bar{u} = \int_A \frac{u da}{A}$ as follows:

$$E = \alpha \tfrac{1}{2} \rho \bar{u}^3 A,$$

and

$$M = \beta \rho \bar{u}^2 A,$$

where α and β are respectively the energy and momentum correction factors. As thus defined these factors are specifically

$$\alpha = \frac{1}{A} \int_A \left(\frac{u}{\bar{u}}\right)^3 da$$

$$\beta = \frac{1}{A} \int_A \left(\frac{u}{\bar{u}}\right)^2 da.$$

The use of these coefficients has been discussed by O'Brien and Johnson (2), who gave some numerical values occurring in practice. The momentum factor may be used in some cases to correct measurements of water flow by the Gibson method (4).

General Relation Between the Coefficients

Let $\frac{u}{\bar{u}} = 1 + z$, and note that by definition $\int_A z da \equiv 0$. On expanding, the factors become

$$\alpha = \frac{1}{A} \int_A (1 + 3z + 3z^2 + z^3) da \equiv 1 + 3I_1 + I_2$$

and

$$\beta = \frac{1}{A} \int_A (1 + 2z + z^2) da \equiv 1 + I_1,$$

where

$$I_1 = \frac{1}{A} \int_A z^2 da \quad \text{and} \quad I_2 = \frac{1}{A} \int_A z^3 da.$$

The only general relation between α and β is thus found to be

$$\alpha - 1 = 3(\beta - 1) + I_2.$$

Since z is a real quantity, z^2 is always positive and therefore I_1 is always positive. I_2 may be either positive or negative, depending on the velocity distribution. In the majority of the practical problems involving flow in pipes, ducts, and channels it will be found that

(a) there are no negative velocities, and

(b) the mean velocity is more than half the maximum velocity,

or, simply, $0 \leqslant u \leqslant 2\bar{u}$. In these cases $|z| \leqslant 1$ and consequently $|z^2| \geqslant |z^3|$, with the result that $|I_1| > |I_2|$. This provides a very useful relation in

checking numerical results. It can further be shown that if the velocity distribution is of a certain kind, which is found in most closed ducts and many open channels, the value of I_2 is negative. All the above relations apply to streams with any shape of cross-section.

The Velocity Distribution in Circular Pipes

As a result of the theoretical contributions of Prandtl and von Karman and the experimental work of Nikuradse, it is possible to predict very closely the velocity distribution in circular pipes with fully developed turbulent flow. A résumé of this subject has been presented by Bakhmeteff (1), and the numerical constants quoted by him will be used here.

In a recent instance it was desired to know the value of α for a particular pipe. This was determined experimentally by taking the following steps. A special pitot tube and supporting and traversing apparatus was designed and constructed. Velocity traverses were made on two mutually perpendicular diameters, taking 17 points on each diameter. Assuming that the distribution measured on a radius could be applied with accuracy to the whole quadrant, the values of the α and β were obtained, integrating by the use of a planimeter. After securing the experimental values, the coefficients were computed using Karman–Prandtl universal velocity distributions. The results were as follows:

	α	β
By experiment	1.044	1.013
By Karman–Prandtl formulae	1.043	1.015

This clearly indicated that unless it is possible to undertake experiments of a high order of accuracy, the values of α and β for circular pipes may be computed with entire satisfaction from an assumed velocity distribution.

Additional Notation

In addition to the symbols used above, the following are also required,

u_m = the maximum velocity, occurring at the centre of the pipe,

u_* = the friction velocity $= \sqrt{\frac{\tau}{\rho}}$,

τ = the shear stress in the fluid at the pipe wall,

r = the radius of the pipe,

x = the distance from the pipe wall divided by the radius,

ν = the kinematic viscosity of the fluid,

k = a linear dimension denoting the scale of the roughness,

R_* = the friction Reynolds number $= \frac{u_* r}{\nu}$,

R = the bulk Reynolds number $= \frac{\bar{u} 2r}{\nu}$,

λ = the pipe friction factor $= \frac{8\tau}{\rho u^2}$,

C_1, C_2, C_3 = numerical constants.

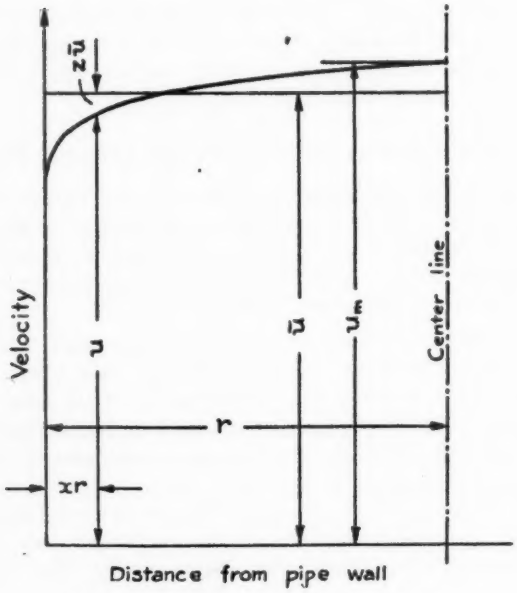

FIG. 1. *Diagram illustrating notation used.*

Derivation of Correction Factors

The dimensionless universal velocity distributions are *(a)* for smooth walled pipes

$$\frac{u}{u_*} = C_1 (\ln x + \ln R_* + C_2) \tag{1}$$

and *(b)* for rough walled pipes

$$\frac{u}{u_*} = C_1 \left(\ln x + \ln \frac{r}{k} + C_3 \right), \tag{2}$$

each of which may be expressed as

$$\frac{u}{u_*} = \frac{u_m}{u_*} + C_1 \ln x . \tag{3}$$

When plotted in this form each velocity distribution curve is identical in shape with every other, the only distinguishing feature being the vertical position, denoted by the value of $\frac{u_m}{u_*}$. If, therefore, the vertical position is known the velocity distribution is completely determined and the energy and momentum correction factors can readily be found.

The mean velocity is obtained by integrating over the area of the circle

$$\frac{\bar{u}}{u_*} = 2 \int_0^1 \left(C_1 \ln x + \frac{u_m}{u_*} \right) (1 - x) dx = \frac{u_m}{u_*} - \frac{3}{2} C_1 . \tag{4}$$

The variation velocity ratio

$$z = \frac{u}{\bar{u}} - 1 = \frac{C_1 u_*}{u}\left(\ln x + \frac{3}{2}\right)$$

and the integrals occurring in the correction factors become

$$I_1 = \frac{1}{A}\int_A z^2 da = 2\left(\frac{C_1 u_*}{\bar{u}}\right)^2 \int_0^1 \left(\ln x + \frac{3}{2}\right)^2 (1 - x)\, dx = \frac{5}{4}\left(\frac{C_1 u_*}{\bar{u}}\right)^2, \quad (5)$$

and

$$I_2 = \frac{1}{A}\int_A z^3 da = 2\left(\frac{C_1 u_*}{\bar{u}}\right)^3 \int_0^1 \left(\ln x + \frac{3}{2}\right)^3 (1 - x)\, dx = -\frac{9}{4}\left(\frac{C_1 u_*}{\bar{u}}\right)^3. \quad (6)$$

Special Treatment of Low Reynolds Numbers

Since the universal velocity distributions do not apply in the region close to the wall, there seemed some doubt as to the validity of integrating in this region, particularly as the velocities pass through zero and negative values and finally become infinite at the wall. As a check on the magnitude of the error that might arise, a better approximation was made in this region by assuming the laminar distribution. Thus

$$\frac{u}{u_*} = R_*\left(x - \frac{x^2}{2}\right) \qquad \text{for } 0 < x < x_1$$

$$\frac{u}{u_*} = C_1(\ln x + \ln R_* + C_2) \qquad \text{for } x_1 < x < 1,$$

where x_1 is the larger of the two roots of the equation

$$x - \frac{x^2}{2} + \frac{C_1}{R_*} \ln \frac{1}{x} = \frac{C_1}{R_*} (\ln R_* + C_2).$$

This required that all integrations be done in two parts, from 0 to x_1 by one formula and from x_1 to 1 by another. The integrals in the laminar portion become polynomials in the powers of x_1. The process was cumbersome, and was carried out only for four discrete values of R_*. The results of these checks showed that above $R_* = 10{,}000$ the error caused by applying Equation (3) over the whole pipe area is not appreciable.

The comparison between the simple formula and the improved approximation is as follows:

R_*	I_1		I_2	
	Univ. dist.	U.D. + laminar	Univ. dist.	U.D. + laminar
200	0.0347	0.0661	−0.0104	−0.0337
500	0.0261	0.0377	−0.0068	−0.0168
2000	0.0181	0.0207	−0.0039	−0.0065
10000	0.0127	0.0132	−0.0023	−0.0028

Conversion to Convenient Forms

In using Equations (5) and (6) to obtain actual values of α and β it may be that other quantities are more readily known than $\frac{C_1 u_*}{\bar{u}}$ which appears.

Below are given a number of convenient substitutions

$$\frac{C_1 u_*}{\bar{u}} = \frac{1}{\left(\ln R_* + C_2 - \frac{3}{2}\right)} \text{ for smooth pipes,}$$

$$= \frac{1}{\left(\ln \frac{r}{k} + C_2 - \frac{3}{2}\right)} \text{ for rough pipes,}$$

$$= C_1\sqrt{\frac{\lambda}{8}} \text{ for both smooth and rough pipes,}$$

$$= \frac{1}{3}\epsilon, \text{ for both smooth and rough pipes, where } \epsilon = \frac{u_m}{\bar{u}} - 1.$$

(This is a term used by Rehboch (3) in expressions for α and β based on the elementary assumption that the velocity vs. area diagram is a straight line. For Rehboch's case $\alpha = 1 + \epsilon^2$ and $\beta = 1 + \frac{1}{3}\epsilon^2$, whereas for the present case $\alpha = 1 + \frac{5}{3}\epsilon^2 - \frac{2}{3}\epsilon^3$ and $\beta = 1 + \frac{5}{9}\epsilon^2$).

It is expected that for engineering use it will be most convenient to refer the coefficients to R, the bulk Reynolds number, for smooth walled pipes, and to λ, the friction factor, for rough walled pipes. Tables I and II have been prepared in this form and the same results are presented in graphical

TABLE I

FOR SMOOTH WALLED PIPES

$\log_{10} R_*$	$\log_{10} R$	α	β
2.301	3.767	1.164	1.066
2.699	4.234	1.096	1.038
3.301	4.918	1.056	1.021
4.000	5.695	1.037	1.013
4.301	6.025	1.031	1.011
4.699	6.459	1.027	1.009
5.000	6.786	1.024	1.008
5.301	7.111	1.021	1.0075
5.699	7.539	1.019	1.0065
6.000	7.861	1.017	1.006
6.301	8.181	1.016	1.0055
6.699	8.605	1.014	1.005
7.000	8.925	1.013	1.0045

TABLE II

FOR ROUGH WALLED PIPES

λ	α	β	λ	α	β
0.010	1.028	1.010	0.030	1.080	1.029
0.012	1.033	1.012	0.0333	1.088	1.033
0.014	1.038	1.014	0.0367	1.097	1.036
0.016	1.044	1.016	0.04	1.105	1.039
0.018	1.049	1.018	0.045	1.117	1.044
0.020	1.054	1.020	0.05	1.129	1.049
0.0225	1.061	1.022	0.055	1.141	1.054
0.025	1.067	1.024	0.06	1.153	1.059
0.0275	1.073	1.027			

form in Figs. 2 and 3. In all the numerical calculations the following values were given to the constants; $C_1 = 2.5$, $C_2 = 2.2$. The bulk Reynolds number is obtained from the friction Reynolds number by the equation

$$R = 2R_*\left(\ln R_* + C_1C_2 - \frac{3}{2}C_1\right),$$

with appropriate corrections for the low Reynolds numbers.

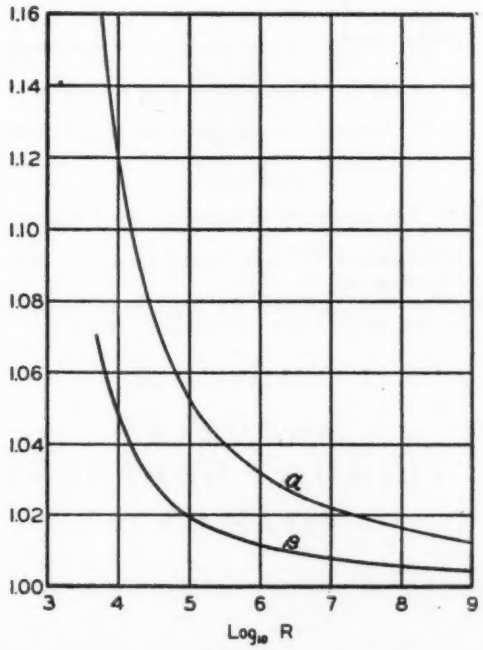

FIG. 2. *Values of α and β against bulk Reynolds number.*

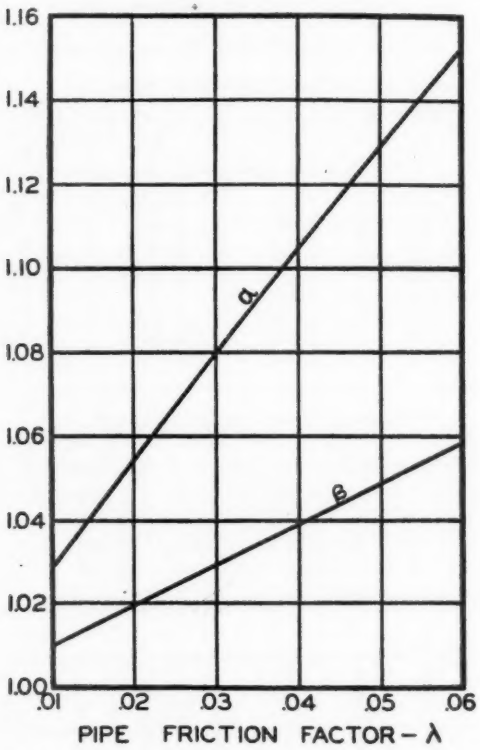

FIG. 3. *Values of α and β against pipe friction factor.*

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VOL. 20, SEC. B. DECEMBER, 1942 NUMBER 12

THE NATURAL OCCURRENCE OF 3-METHOXY-PYRIDINE[1]

BY RICHARD H. F. MANSKE[2]

Abstract

A volatile base, identified as 3-methoxy-pyridine, has been isolated from *Thermopsis rhombifolia* (Nutt.) Richards and from *Equisetum arvense* L.

The alkaloids of *Thermopsis rhombifolia* (Nutt.) Richards have been under investigation by the author for some time, but owing to the difficulties of crystallization of some of the fractions it is expected that publication will be further delayed.

In the meantime it seems advisable to record the isolation of a small amount of a very volatile base, which has been identified as 3-methoxy-pyridine. It constituted about 3% of the total bases, which in turn were present to the extent of 0.25% in the dried whole plant exclusive of roots. Its positive identification necessitated the synthesis of an authentic specimen because the properties of its derivatives deviated considerably from the recorded values. It was ultimately purified as its picrate (m.p. 139° C.)*, which had not previously been prepared. The base regenerated from the latter was distilled *in vacuo* and from this the double mercurichloride and the platinichloride were prepared. Meyer (4) first prepared 3-methoxy-pyridine by treating 3-hydroxy-pyridine with diazomethane, and he recorded the melting points of the mercurichloride and of the platinichloride as 110° C. and 182° C. respectively. Later, Koenigs, Gerdes, and Sirot (2) prepared 3-methoxy-pyridine by heating 3-bromo-pyridine with sodium methylate in methanol at 150° C. for 48 hr. They recorded that the platinichloride melts at 269° C. and remarked that it is very sparingly soluble.

When the author repeated the latter preparation of 3-methoxy-pyridine, it was found that the product contained a considerable amount of bromine, and no pure picrate could be isolated from it. Ciamician and Dennstedt (1) had already shown that 3-bromo-pyridine could be reduced to pyridine by means of zinc and hydrochloric acid. The mixture of methoxy- and bromo-pyridine was therefore reduced with this reagent and the fraction of the regenerated base that boiled above 150° C. was redistilled *in vacuo*. It was then free of bromine and the picrate prepared from it melted at 139° C. either

[1] *Manuscript received September 29, 1942.*

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[2] *Chemist.*

* *All melting points are corrected.*

alone or in admixture with the specimen of natural origin. The mercurichloride and the platinichloride melted at 120° C. and 194° C. respectively, and no depression was observed in these melting points when the synthetic and natural products were mixed. The high melting point (269° C.) of the platinichloride of 3-methoxy-pyridine recorded by Koenigs and co-workers can now be explained. They prepared their derivative from a product that undoubtedly contained bromine, and it has now been observed that the platinichloride of 3-bromo-pyridine is much less soluble than that of 3-methoxy-pyridine. A specimen of the former prepared from pure 3-bromo-pyridine melted in its water of crystallization at about 200° C. It then resolidified at 210 to 220° C. and finally melted at 268 to 269° C. Ciamician and Dennstedt (1) had already prepared 3-bromo-pyridine platinichloride and showed that it has two molecules of water of crystallization. It is obvious therefore that Koenigs, Gerdes, and Sirot actually had in their possession the platinichloride of 3-bromo-pyridine instead of that of 3-methoxy-pyridine in spite of the almost perfect platinum analysis that they recorded for the latter.

In the course of an examination of the bases of *Equisetum arvense* L. the author in collaboration with L. Marion (3) obtained a few milligrams of a very volatile base (more volatile than nicotine, from which it was separated by fractional distillation) that yielded a picrate melting at 135° C. A single analysis indicated that the picrate was that of a base, C_6H_7ON. The crude picrate from *T. rhombifolia* also melted at 135 to 136° C. and a mixture of the two substances melted at this temperature. When the *Equisetum* base picrate was recrystallized once from methanol it melted at 137 to 138° C. and this when admixed with pure 3-methoxy-pyridine picrate also melted at 137 to 138° C. There is therefore no question but that at least one species of *Equisetum* also contains this, perhaps the simplest of natural bases.

Experimental

Isolation of 3-Methoxy-pyridine

The mixture of total ether soluble bases from *T. rhombifolia* was slowly heated to 150° C. *in vacuo* (1 mm.), and the distillate, which was collected in a cooled receiver, was redistilled. The fraction boiling at 40 to 60° C. (1 mm.) was dissolved in hot methanol and treated with twice its weight of picric acid also dissolved in hot methanol. The picrate, which crystallized almost at once (m.p. 135 to 136° C.), was recrystallized twice more from hot methanol. It was then obtained in large stout prisms that melt sharply at 139° C., and further recrystallization failed to alter this value. Found: C, 42.81, 42.53; H, 2.96, 3.07; N, 16.41%. Calc. for $C_{12}H_{10}O_8N_4$; C, 42.60; H, 2.96; N, 16.57%.

The free base was obtained by suspending the picrate in an excess of aqueous sodium hydroxide and shaking with ether. The ether solution was washed with a little water, dried over sodium hydroxide, and the solvent distilled off. The residue was distilled *in vacuo* and boiled at 40° C. (1 mm.). The colourless base thus obtained had an odour much like that of pyridine

and showed a slight blue fluorescence. Found: C, 66.09, 66.02; H, 6.39, 6.40; OMe, 27.20%. Calc. for C_6H_7ON: C, 66.05; H, 6.42; OMe, 28.18%.

The *mercurichloride* was prepared by adding mercuric chloride in methanol to a methanolic solution of the base containing an excess of hydrochloric acid. Some of the methanol was evaporated and hot water added. The double salt then crystallized in large brilliant prisms that melted at 120° C. Recrystallization from hot water, in which it is moderately soluble, did not alter this value.

The *platinichloride* crystallized in golden plates when a methanolic solution of platinic chloride was added to a solution of the base in methanolic hydrochloric acid. It melted sharply at 194° C.

The *aurichloride* crystallized slowly from water in pale yellow fragile needles that melted at 176° C.

Synthesis of 3-Methoxy-pyridine

A mixture of 3-bromo-pyridine (28 gm.) and sodium methylate (4.6 gm. of sodium in 40 cc. of methanol) was heated in a steel autoclave at 150 to 160° C. for 48 hr. Most of the methanol was evaporated from the mixture, which was then extracted with ether. The residue from the ether extract was twice distilled *in vacuo* (b.p. about 50° C. (1 mm.)). It contained large quantities of halogen and a picrate prepared in methanol melted indefinitely at 115 to 130° C.

The total base was then dissolved in dilute hydrochloric acid and gently boiled for four to five hours with an excess of granulated zinc. The base was recovered by adding an excess of aqueous sodium hydroxide to the filtered solution and extracting with ether. The residue from the ether extract was distilled at atmospheric pressure until the temperature of the vapour rose to 150° C. The remainder was then distilled and redistilled *in vacuo*. There was thus obtained 6 gm. of water-white 3-methoxy-pyridine, which gave a negative test for halogen.

The picrate melted at 137 to 138° C. and when recrystallized from hot methanol it melted sharply at 139° C. either alone or in admixture with a specimen of the picrate obtained from *T. rhombifolia*.

The mercuri-chloride and the platinichloride were prepared as above described. Their melting points were the same as those of the corresponding derivatives of natural origin, and the appropriate mixtures showed no depression in melting points.

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TASTE DIFFERENCES IN COMPOUNDS HAVING THE NCS LINKAGE[1]

By C. Y. Hopkins[2]

Abstract

A group of compounds consisting of thion-thiazolidines, thion-oxazolidines, and a thion-thiazoline were found to be tasteless to some persons and very bitter to others. The three oxo-thiazolines that were examined did not exhibit this difference, being tasteless to all the subjects. A number of these compounds have been prepared for the first time, and their synthesis is described.

Taste tests were conducted on other sulphur compounds and it is concluded that the "dual taste reaction" accompanies the —NH—C(=S)— grouping.

Introduction

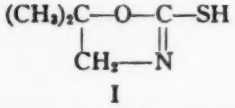
During a study of the bitter principle of the seed of *Conringia orientalis* (3), the author isolated the substance 2-mercapto-5,5-dimethyl-oxazoline and found it to be tasteless. Shortly afterwards, Mr. D. C. Caplan, working in the same laboratory, was bottling a small quantity of the substance and remarked that it had an intensely bitter taste.

It was apparent that this compound has the peculiar characteristic found by Fox in phenylthiourea (1), viz., that it is tasteless to some persons and very bitter to others. The fact was confirmed by conducting taste tests with a number of individuals.

Although Fox examined a large number of thiourea derivatives and found that nearly all had the dual taste reaction, the present instance is the first in which it has been found in a nitrogen ring compound. Accordingly, taste tests were carried out with several compounds prepared during the previous investigation and on others of similar constitution.

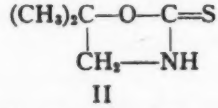
Nomenclature

2-Mercapto-5,5-dimethyl-oxazoline is very probably tautomeric and so may exist in the mercapto or thion form. The thion formula (II) is preferred.



I

2-Mercapto-5,5-dimethyl-oxazoline



II

2-Thion-5,5-dimethyl-oxazolidine

[1] *Manuscript received August 13, 1942.*

Contribution from the Division of Chemistry, National Research Laboratories, Ottawa, Canada. Issued as N.R.C. No. 1092.

Presented before Section III of the Royal Society of Canada, May 24, 1939.

[2] *Chemist.*

Taste Tests with Thiazo and Oxazo Compounds

Table I shows the results of taste tests by the author (C.Y.H.) and by Mr. Caplan (D.C.C.) with a number of cyclic oxygen and sulphur compounds whose taste has not hitherto been reported.

Tests were ordinarily made by tasting the crystals of each substance. The compounds in Table I have a slight solubility in water, being similar to phenylthiourea in this respect. Some trials were made with dilute solutions but no advantage was found in using this method.

Substances having the dual taste reaction are classed as positive, while those that taste the same to all individuals are referred to as negative.

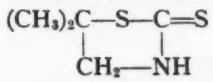
TABLE I

TASTE TESTS WITH THIAZO AND OXAZO COMPOUNDS

Substance	Taste sensation		Dual taste reaction
	(C.Y.H.)	(D.C.C.)	
2-Thion-5,5-dimethyl-oxazolidine II	Tasteless	Very bitter	+
2-Thion-5,5-dimethyl-thiazolidine	Tasteless	Very bitter	+
2-Thion-5-methyl-oxazolidine	Tasteless	Very bitter	+
2-Thion-5-methyl-thiazolidine	Tasteless	Very bitter	+
2-Thion-thiazolidine	Tasteless	Very bitter	+
2-Oxo-4,5-dimethyl-thiazoline	Tasteless	Tasteless	−
2-Thion-4,5-dimethyl-thiazoline	Tasteless	Very bitter	+
2-Oxo-4-methyl-thiazoline	Tasteless	Tasteless	−
2-Oxo-4-methyl-5-bromo-thiazoline	Tasteless	Tasteless	−
2-Thion-4,4-dimethyl-oxazolidine	Tasteless	Very bitter	+

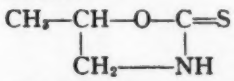
The taste tests were confirmed by repeating the tests with a group of individuals, some of whom were tasters and some non-tasters, as determined by their reaction to phenylthiourea. Upon tasting the substances listed in Table I, the "tasters" experienced the same sensations as did Mr. Caplan, while the non-tasters found all the materials to be tasteless.

Considering substance II, it was found that replacement of the oxygen in the ring by sulphur did not destroy the dual taste reaction:

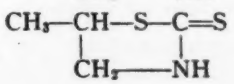


2-Thion-5,5-dimethyl-thiazolidine

The corresponding pair of compounds with one methyl group had the same characteristic:

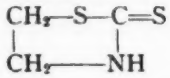


2-Thion-5-methyl-oxazolidine



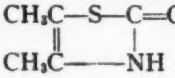
2-Thion-5-methyl-thiazolidine

Elimination of the substituent methyl group had likewise no effect on the peculiar taste reaction:



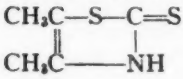
2-Thion-thiazolidine

However, on replacing the thion sulphur by oxygen, the bitterness disappeared and this substance was tasteless to all the subjects:



2-Oxo-4,5-dimethyl-thiazoline

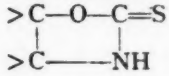
The corresponding thion-thiazoline was bitter to the so-called tasters and tasteless to the non-tasters:

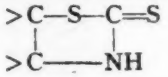


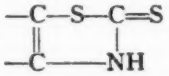
2-Thion-4,5-dimethyl-thiazoline

2-Oxo-4-methyl-thiazoline was found to be tasteless to all the subjects.

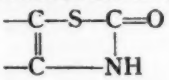
Summarizing the data, it is observed that the following groupings, with various substituents, gave the dual taste reaction,







while the oxo-thiazoline derivatives did not,



The grouping —NH—C=S appears to be connected with the unusual taste characteristic and the arrangement —NH—C—S— apparently does not give this effect.

Taste Tests with Various Sulphur Compounds

Further tests were carried out with common substances as shown in Table II.

TABLE II

TASTE TESTS WITH OTHER —N—C=S COMPOUNDS

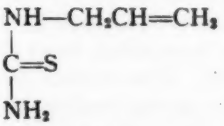
Substance	Taste sensation		Dual taste reaction
	(C.Y.H.)	(D.C.C.)	
Phenylthiourea	Tasteless	Very bitter	+
Thioacetamide	Almost tasteless	Very bitter	+
Thioacetanilide	Tasteless	Very bitter	+
Isodithiocyanic acid	Tasteless	Very bitter	+
Allylthiourea	Momentary faint bitterness	Very bitter and persistent	+
Thiobarbituric acid	Faint taste	Faint taste, not bitter	−

The simplest substance having the NHCS group is thioformamide, $HC(=S)-NH_2$. It has been described by Willstätter as very bitter. This substance was not readily available. Thioacetamide, however, was found to be very bitter to the tasters and almost tasteless to the non-tasters. Thioacetamide is very soluble in water and it is evident, therefore, that the bitter taste is not dependent upon the degree of solubility, since it was virtually tasteless to certain persons. Although thioacetamide is a very common material, this taste difference has not hitherto been observed. It is remarkable also that Cohn, author of the exhaustive work on taste, "Die Organischen Geschmackstoffe", failed to realize that the same substance might taste differently to different persons. He actually reported one or two such cases but stated that one observer must have been mistaken.

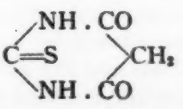
In thioacetanilide $CH_3 . C(=S) . NHC_6H_5$ one of the amino hydrogens is substituted but the taste difference remains. Fox (1) found that if both hydrogens are substituted, as in tetramethyl thiourea, the taste difference disappears.

On the basis of these observations, it is concluded that the dual taste reaction accompanies the grouping $-NH-C(=S)-$. Some 20 thiourea derivatives prepared by Fox and containing this grouping were shown to taste differently to different persons and 11 of the substances described above exhibited the same characteristic. The reaction of tasters to isodithiocyanic acid, NH . CS . NH . CS, confirms this theory.

All compounds having the $-NH-C(=S)-$ group do not necessarily have the dual taste reaction. Various substituents may influence the taste so as to overshadow the effect of the NHCS group. Thus thiourea itself is not bitter but sour to all persons (1). Allylthiourea has a momentary bitterness even to the so-called non-tasters (Table II). The unsaturation appears to intensify the bitterness, as shown also in crotylthiourea (1). On the other hand thiobarbituric acid is exceptional in that it has no bitter taste to persons of either group. It is possible that the CO groups overcome the influence of the CS group towards bitterness.



Allylthiourea



Thiobarbituric acid

Preparation of Compounds Listed in Table I.

2-Thion-5,5-dimethyl-oxazolidine. (3).

2-Thion-5,5-dimethyl-thiazolidine. A sample of this material was kindly supplied by Dr. H. A. Bruson.

2-Thion-5-methyl-oxazolidine. Twenty-five grams of 1-aminopropanol-2 and 38 gm. of carbon disulphide were mixed gradually with cooling. A solution of 28 gm. of potassium hydroxide in 20 cc. of water and 150 cc. of ethanol was added and the mixture was refluxed for 2½ hr. After distilling off most of the alcohol, water was added, and the solution was extracted with chloroform. The material obtained from the extract was recrystallized several times from benzene. When pure it melted at 72 to 73°.* Found: C, 41.03; H, 6.17; S, 27.43%. Calc. for C_4H_7ONS: C, 41.03; H, 6.03; S, 27.38%. It is soluble in alcohol, ether and benzene, moderately soluble in water, and insoluble in hexane.

2-Thion-5-methyl-thiazolidine. Gabriel (2, p. 814) prepared this substance from β-bromopropylamine and carbon disulphide and gave its melting point as 88 to 89° (uncorrected). The material obtained in the present work melted at 92 to 93° uncorrected (93 to 94° corrected). Found: C, 36.21, 36.12; H, 5.23, 5.15; N, 10.21, 10.32%. Calc. for $C_4H_7NS_2$: C, 36.07; H, 5.30; N, 10.52%. The procedure was as follows: twenty-five grams of 1-aminopropanol-2 was mixed while cooling with 50 gm. of carbon disulphide. A solution of 28 gm. of potassium hydroxide in 20 cc. of water and 150 cc. of ethanol was added and the mixture was refluxed for six hours. The alcohol was distilled off and the residue was acidified with 1 : 1 sulphuric acid. The crystals (largely inorganic) were filtered off and leached with hot benzene. The benzene solution was concentrated and cooled, whereupon the product crystallized out. The yield was poor.

2-Thion-thiazolidine. This substance was prepared from 1-aminoethanol-2 and carbon disulphide according to the method of Knorr and Rössler (5).

2-Oxo-4,5-dimethyl-thiazoline. A mixture of 5 gm. of 3-chlorobutanone-2, 6 gm. of potassium thiocyanate, 1.5 gm. of sodium bicarbonate and 75 cc. of water was allowed to stand for three days. The solid product was filtered off and recrystallized from benzene. It melted at 149 to 150°. Found: C, 46.32; H, 5.65; N, 10.87; S, 24.72%. Calc. for C_5H_7ONS: C, 46.49; H, 5.46; N, 10.84; S, 24.82%. It is soluble in alcohol, moderately soluble in ether, chloroform and benzene, slightly soluble in water, and insoluble in hexane.

2-Thion-4,5-dimethyl-thiazoline. Fifteen grams of 3-chlorobutanone-2 and 12 gm. of ammonium dithiocarbamate were mixed in 100 cc. of alcohol. Considerable heat was evolved. The mixture was allowed to stand 12 hr. and was then refluxed for one hour. Water was added, most of the alcohol was distilled off, and the solution was cooled. The crystals that formed on standing were recrystallized from benzene; the product melted at 166 to 168°.

* *Melting points are corrected.*

Found: N, 9.54%. Calc. for $C_5H_7NS_2$: N, 9.66%. It is soluble in alcohol and ether, moderately soluble in benzene, difficultly soluble in water, and insoluble in hexane.

2-Oxo-4-methyl-thiazoline (oxymethylthiazole). This substance was prepared from choroacetone and potassium thiocyanate by the method of Tcherniac (7). It is not necessary to let the reaction proceed for 10 days, however, since the product may be extracted after two days with only a slight sacrifice in yield. Further time is saved by extracting with chloroform instead of ether, since the material is more soluble in the former. Recrystallization is best done in benzene with cooling to 0° C.

When the reaction was carried out in alcohol instead of water, a substance was obtained that was insoluble in benzene. After recrystallization from alcohol it melted at 193°. It was not investigated further.

2-Oxo-4-methyl-5-bromo-thiazoline. This substance was prepared by the method of Ochiai and Nagasawa (6, p. 1471). It crystallizes in silky needles, m.p. 150° C., and is soluble in alcohol and ether, slightly soluble in benzene and chloroform, and insoluble in hexane.

2-Thion-4,4-dimethyl-oxazolidine. Thirty grams of 2-amino-2-methyl-1-propanol and 38 gm. of carbon disulphide were mixed gradually with cooling. A solution of 28 gm. of potassium hydroxide in 20 cc. of water and 150 cc. of ethanol was added and the mixture was refluxed for 2½ hours. After distilling off most of the alcohol, 150 cc. of water was added and the solution was extracted with chloroform. The material obtained from the extract was recrystallized from benzene. When pure it melted at 123 to 125° C. Found: N, 10.80%. Calc. for C_5H_9ONS: N, 10.69%. It is soluble in alcohol, ether, and benzene, moderately soluble in water, and insoluble in hexane.

Acknowledgment

The author is indebted to Mr. A. E. Ledingham for his kindness in performing the analyses.

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SOME SOURCES OF VITAMIN C IN ALBERTA[1]

By Hilda K. Waagen[2] and L. B. Pett[3]

Abstract

Some fruits and vegetables grown in Alberta were investigated as sources of Vitamin C, and the loss occasioned by cooking was determined. Losses due to oxidation during cooking were compared with those due to extraction by the cooking water. Both cause considerable loss of vitamin C from the vegetable pulp; it would therefore be more economical always to use the juice from the vegetables. The vitamin C content of potatoes varies with the season, the peak being reached in September and just before the first frost occurs, and being followed by a steady decrease during storage.

Introduction

Two main factors determine the quantity of vitamin C in the diet, namely; the food, or the source, and the method of preparation for consumption. These factors are of equal importance and their value depends on several different conditions. Thus, as is generally known, the vitamin C content of vegetables and fruits varies with the season, variety, soil conditions, and locality. Similarly the mode of preparation is dependent on the facilities, the type of cooking, and the cook's knowledge regarding means of preservation of the vitamin C.

Extensive surveys have been carried out in many countries to ascertain the best and cheapest sources of vitamin C (2–5, 7, 8, 12). Since the ascorbic acid contents of vegetables and fruits vary under different conditions, the values obtained in other countries cannot always be accepted as criteria of the values for Canadian products. It is thus desirable that surveys of Canadian vegetables and fruits should be carried out.

In this paper an indication is given of the vitamin C content to be expected in certain vegetables and fruits. The content of the vitamin in a few samples of numerous different fruits and vegetables, rather than in many samples of two or three, was determined. The samples were tested before and after cooking, to determine the loss of vitamin C during food preparation. Also included in the paper are the values obtained with foods tested during the year's survey of the Athabasca Hall dietary (11).

Experimental

A survey of the vitamin C content of locally grown vegetables and fruits was carried out. All vegetables were tested as soon after harvesting as possible, usually on the same day. If any had to be left over, they were

[1] *Manuscript received in original form August 3, 1942, and, as revised, September 1, 1942. Contribution from the Department of Biochemistry of the University of Alberta, Edmonton, Alta., with financial assistance derived from a grant to Prof. G. Hunter from the Associate Committee on Medical Research of the National Research Council of Canada. This work was included in a thesis for the degree of Master of Science in the University of Alberta.*

[2] *Formerly Research Assistant, 1939–41.*

[3] *Director of Nutrition Services, Department of Pensions and National Health, Ottawa. At the time, Lecturer in Biochemistry, University of Alberta.*

put in the icebox at 5° C. until ready for use. The large vegetables, such as potatoes, turnips, carrots, etc., were cut into two equal parts, one of which was tested in the raw condition for vitamin C, and the other was first cooked, then tested. The vegetables were boiled in water in sufficient quantity just to cover them until done, overcooking being avoided.

The method of preparation of the tissue extract and the titration were the same as in previous work (11).

During the survey of the Athabasca Hall dietary, the vitamin C contents of many different foods were determined. The list is included in this paper as a supplement to the values obtained on locally grown vegetables. Description of the method of the survey was given in a preceding paper (11).

Results

Table I was compiled from results obtained in determinations carried out on vegetables and fruits grown in the University of Alberta gardens.

The loss of vitamin C during cooking varies greatly; it is considerable in some vegetables, small in others, and the occasional vegetable shows an apparent increase. The percentage loss is less in fruits owing to their acidity (1). In the cooking of leafy vegetables such as kale, broccoli, and beet tops there is a tendency for the vitamin C to be largely destroyed. There is also high loss in celery and onions; the reason for this is not apparent.

The vitamin C content varies with the variety of the vegetable and with the individual plants of the same variety. The average value and the range give the best indication of the content. Of the raw vegetables, parsley was found to have the highest vitamin C value; rhubarb, the lowest. Of the cooked vegetables and fruits, strawberries have the highest vitamin C content, with black currants a close second. Broccoli, kale, Brussel sprouts, and asparagus tips are good sources of the vitamin. Since potatoes are so universally consumed during the winter and summer, and in large amounts, they are good sources.

It is noted that the vitamin C content of asparagus tips is double that of the stalks; this agrees with the results obtained by Olliver (7). Wheeler *et al.* (13) note that the vitamin C content of the outer leaves of the lettuce is slightly higher than that of the inner. This is in agreement with our values for lettuce.

There has been much controversy concerning the cause of the apparent increase of vitamin C content on heating some vegetables. Reedman and McHenry (9) maintain that the vitamin is present as a protein conjugate, which is broken down on heating to give free ascorbic acid. Olliver (7) suggests that there may be incomplete extraction from the raw vegetables, especially woody ones, which are difficult to grind. On cooking, the tissues are softened, and the extraction of vitamin C is more complete, with resultant higher estimates. Van Eekelen (10) suggests that the increase after cooking is due to the destruction of the ascorbic acid oxidase. The latest suggestion

made by Mack and Tressler (6) is that the apparent increase is caused by the decomposition of dehydro ascorbic acid into physiologically inactive but strongly reducing products. The question is far from answered. But as

TABLE I

PERCENTAGE LOSS OF VITAMIN C IN COOKING. VITAMIN C EXPRESSED IN MG. PER 100 GM. WET WEIGHT

Vegetable or fruit	Variety	No. of samples	Average no. mg. Vitamin C, and range		Average loss, %
			Raw	Cooked	
Asparagus—tips	Mary Washington	4	52 (35 – 69)	32 (18 – 51)	38.0 (26.5 – 65.8)
—stalks		4	22 (11 – 32)	16 (10 – 24)	24.3 (20.6 – 57.2)
Beans	Yellow Wax	1	29	24	18
	Green String	1	37	22	39
Beets—tops	Detroit Dark Red	1	44	19	56
	Crosby's Egyptian	1	40	19	53
	Lutz's Greenleaf	1	45	20	55
—roots	Detroit Dark Red	1	14	6.1	57
	Crosby's Egyptian	1	13	15	
	Lutz's Greenleaf	1	4.7	6.6	
Broccoli	Sprouting	3	94 (83 – 102)	30 (28 – 32)	
Brussels Sprouts		2	81 (Both)	38 (30 – 46)	
Cabbage	Late Green	2	30 (29 – 31)	20 (Both)	
Carrots	Red Cored Chantenay	1	5.4	2.5	
	Streamliner	2	14 (12 – 17)	7 (4 – 10)	
Celery	White	2	11 (10 – 11)	3.1 (2.3 – 3.8)	70.5
	Pink	1	10	2.4	76.0
Cherry	Brooks Sand Cherries	1	10	8.6	16.3
Cherry plum	Sapa	1	10	11	9.3 inc.
	Tom Thumb	1	6.5	9.3	43.2 inc.
Corn		2	8.9 (8.5 – 9.3)	5.8 (4.7 – 7.0)	34.8 (17.9 – 49.6)
Chives		1	36	—	—
Crabapples	Adam (early)	1	6.2	4.1	33.8
	Protosh (late)	1	9.7	10.2	5.8 inc.
Cress		1	35	—	—
Currants	Red	1	27.0	26	3.6
	Black	1	88	62	29.7

TABLE I—*Concluded*

PERCENTAGE LOSS OF VITAMIN C IN COOKING. VITAMIN C EXPRESSED IN MG. PER 100 GM. WET WEIGHT—*Concluded*

Vegetable or fruit	Variety	No. of samples	Average no. mg. vitamin C, and range		Average loss, %
			Raw	Cooked	
Kale		1	77	27	64.5
Marrow	Vegetable Marrow	1	9.6	6.1	36.7
Onion	Welsh Winter	1	15	2.9	81.0
Parsley	Evergreen	1	135	—	—
Peas		2	11 (Both)	17 (Both)	53.6 inc. (53.3 – 54.7)
Plums	Selected Seedling Mammoth	1 1	8.3 3.7	9.8 2.8	18.0 inc. 23.9
Potatoes	Netted Gem	4	23 (22 – 24)	18 (17 – 20)	18.5 (11.0 – 25.0)
Radishes	French Breakfast	6	14 (10 – 19)	—	—
Raspberries		1	23	16	27.8
Rhubarb	Macdonald Red	3	1.4 (0.6 – 2.3)	0.4 (0.3 – 0.6)	71.4 (27.8 – 87.9)
Squash	Scallop	1	17	12	29.8
Strawberries	B.C.	1	64	63	1.6
Tomatoes		3	12 (8.4 – 14)	—	—
Turnips	White Milan	2	17 (13 – 21)	6.7 (6.6 – 6.8)	61.5 (50.9 – 68.0)
	White	2	18 (17 – 20)	8.6 (8.6 – 8.6)	53.0 (48.5 – 56.1)
Onions					
bulb		14	21 (8.5 – 39)		
green shoot		14	48 (20 – 97)		
Head lettuce					
Outer leaf		4	11 (5.7 – 22)		
Inner		4	7.7 (1.9 – 16)		
Heart		4	5.4 (0.6 – 12)		

different investigators do not always find the same vegetables showing an increase after heating, it occurs to us that this phenomenon may be manifested by different vegetables in different localities and that it may depend on factors as yet unsuspected.

The vitamin C content of the vegetables and fruits listed was also calculated on a dry weight basis. These are of little practical importance, for it is difficult to visualize our normal consumption of vitamin C in terms of dry weight figures.

Comparison of Cooking Losses due to Oxidation and due to Solution

Tables II and III show a comparison of percentage losses by heat or oxidation, and by solution in the cooking water. Those vegetables showing a loss are listed in Table II, and those showing an apparent gain are listed in Table III.

TABLE II

VEGETABLES AND FRUITS SHOWING LOSS OF VITAMIN C AFTER COOKING. COMPARISON OF LOSSES DUE TO OXIDATION AND DUE TO SOLUTION IN BOILING WATER

Vegetable or fruit	Lost in the boiling water, %	Loss due to oxidation, %	Total loss from pulp, %
Asparagus—tips	33.7	32.1	65.8
—stalks	22.1	35.1	57.2
Beans—string	18.9	20.4	39.3
Black currants	14.5	15.2	29.7
Beet tops	9.8	43.1	52.9
	9.6	45.7	55.3
	5.9	50.7	56.6
Carrots	4.9	57.7	62.6
	2.1	37.6	39.7
Celery—white heart	4.4	74.5	78.9
stalk	4.8	56.8	61.6
—pink	1.4	74.6	76.0
Corn	10.4	7.5	17.9
	7.3	42.2	49.5
Crabapples—Adam	7.6	26.1	33.7
Broccoli	26.1	43.1	69.2
	43.4	23.5	66.9
	40.3	28.7	69.0
Brussels sprouts	34.3	9.0	43.3
	41.4	21.1	62.5
Potatoes	14.1	8.0	22.1
	23.0	2.0	25.0
Vegetable marrow	22.3	14.4	36.7

TABLE III

VEGETABLES SHOWING APPARENT INCREASE IN VITAMIN C CONTENT AFTER COOKING

Vegetable or fruit	Mg. vitamin C/100 gm. wet wt.		Mg. vitamin C in water from 100 gm. cooked vegetables	Apparent increase vitamin C in cooked vegetables plus water, %
	Raw	Cooked		
Beans—Wax	29	24	9.1	14
Cabbage	31	20	12	3
	29	20	15	21
Plums—Mammoth	3.7	2.8	1	3
Potatoes	22	19	4.4	6
	24	20	5.9	8
Raspberries	23	16	11	17
Sand cherries	10	8.6	10	86
Scallop squash	17	12	5.1	0.6
Turnips—White				
centre	20	8.6	18	33
outside	17	8.6	21	74
Cherry plum				
Sapa	10	11	14	150
Tom Thumb	6.5	9.3	11	212
Crabapples—Protosh	9.7	10	0.3	6
Peas	11	17	7.3	121
	11	17	5.0	100
Plums—Selected Seedling	8.3	9.8	3.1	56

In Table II, in the first group of three vegetables the loss of vitamin C is about equally due to oxidation and to solution in the cooking water. The results obtained with the second group show oxidation to be the predominating factor in destruction of the vitamin. In the last group, the greater loss occurs through extraction of the vitamin by the cooking water, though the first sample of broccoli is an exception to this. Olliver (8) maintains that the disappearance of vitamin C occurs chiefly by solution in the water, the actual loss depending on the extent of cooking and the amount of water used. These figures indicate that considerable waste can occur through the procedure of discarding the water from vegetables (this is not done with fruits), but whether that or oxidation is the greater factor in the loss of the vitamin seems to depend on which vegetable is considered. It would indeed be more economical to use the water in which the vegetables are cooked.

In Table III the first eight vegetables and fruits listed show increase in content only when the values for the pulp plus the water are taken together. The last four show an increase in the vitamin C content of the cooked vegetable or fruit over that of the raw without including the vitamin C contained in the juice. The juice of fruits is used, so no loss is entailed. The cooking

water from turnips and cabbages contains a large proportion of the available vitamin C, as does also that from peas. As noted before, this apparent increase may be due to one of several reasons. These factors may also be active during the cooking of the vegetables listed in Table II. If this be so, oxidation must be playing a still larger part in the destruction of vitamin C than is indicated by the figures.

TABLE IV

VITAMIN C CONTENT OF VEGETABLES AND FRUITS TESTED OVER THE PERIOD OF 13 MONTHS. ARRANGED IN ORDER OF DECREASING AVERAGE VITAMIN CONTENT

Food	Preparation	No. of samples	Range	Mg. ascorbic acid per 100 gm. wet weight. Average
Strawberries	Raw	4	77 – 93	84
Grapefruit	Raw	19	37 – 109*	68*
Oranges	Raw	65	16 – 61†	36†
Cauliflower	Steamed	3	24 – 27	26
Cabbage	Raw	9	13 – 32	22
Tomatoes	Raw	33	5 – 29	18
Tomatoes	Canned	6	10 – 25	17
Cabbage	Steamed	6	8 – 28	14
Turnips	Steamed	6	6 – 21	13
Raspberries	Raw	4	8 – 17	12
Parsnips	Steamed	7	4 – 14	8
Bananas	Raw	21	3 – 14	8
Cherries	Raw	4	4 – 12	8
Potatoes	Steamed	130	0 – 35	7
Peaches	Raw	8	2 – 18‡	6‡
Beets	Steamed	5	3 – 13	6
Cucumbers	Raw	2	5 – 6	6
Pineapple	Canned	7	2 – 10	6
Cherries	Canned	2	4 – 5	5
Beets	Pickled	4	2 – 11	4
Peas	Canned	11	2 – 9	4
Lettuce	Raw	5	2 – 7	4
Rhubarb	Stewed	3	2 – 4	4
Apricots	Canned	7	2 – 5	3
Pears	Canned	10	0 – 10	3
Celery	Raw	4	2 – 4	3
Carrots	Steamed	23	1 – 7	3
Peaches	Canned	8	0 – 4	2
Corn	Canned	12	0 – 6	2
Grapes	Raw	3	1 – 5	2
Beans (string)	Canned	9	0 – 4	2
Apples	Raw	25	1 – 4§	2§
Celery	Steamed	6	1 – 2	1
Plums	Stewed	4	All	1

Vitamin C is expressed in milligrams per fruit, not per 100 gm., in the cases of grapefruit, oranges, peaches, and apples.

** The average volume of juice in a grapefruit is 160 cc., or 42 mg. ascorbic acid per 100 cc. juice (average).*

† The average volume of juice in an orange is 75 cc., or 48 mg. ascorbic acid per 100 cc. juice (average).

‡ Av. wt. of peaches tested = 110 gm., or 5.5 mg. ascorbic acid per 100 gm. peach (average).

§ Av. wt. of apples tested = 150 gm., or 1.7 mg. ascorbic acid per 100 gm. apple (average).

Dry weight figures are also available.

Vitamin C Content of Vegetables and Fruits Tested During Athabasca Hall Survey

Table IV lists the values obtained with fruits and vegetables included in the Athabasca Hall dietary throughout 13 months—November, 1939, to November, 1940, inclusive. This table also indicates available sources of vitamin C in Alberta other than local ones (see also Table I).

As shown, fresh strawberries contain the largest amount of vitamin C, but they are available only during the summer months; thus they are not a good year round source. The consumption of oranges and grapefruit is an excellent means of obtaining vitamin C both winter and summer, and, as shown in a previous paper (11), they should certainly be included in the winter diet. Cauliflower, cabbage, turnips, and parsnips are good sources and are available in this country throughout winter and summer. Potatoes form a solid basis for vitamin C nutrition, as they are used regularly in the diet and in large proportion, especially by the poorer classes.

Seasonal Variation of Vitamin C Content of Vegetables

The vitamin C content of potatoes shows a definite seasonal variation, with the values increasing to a peak in September (Table V) or when the potatoes are mature. In Table VI also the highest value is that obtained in September, just before the first frost occurs; the content then drops steadily until at the end of February it is about one-third of the highest value. After September, any further loss is due to storage.

TABLE V

SEASONAL VARIATION OF THE VITAMIN C CONTENT OF SOME FOODS TESTED DURING ATHABASCA HALL SURVEY

Food	Vitamin C												
	Nov.	Dec.	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.
Potatoes (steamed)*	4.3	4.6	2.4	3.3	2.9	1.7	6.1	5.3	7.8	16.0	17.0	16	9.2
Oranges†	33	32	50	47	33	28	40	42	31	32	32	43	29
Grapefruit†	56	98	96	82	66	85	50	57	59	54	—	—	43

* *Vitamin C expressed in mg. per 100 gm.*
† *Vitamin C expressed in mg. per fruit.*

After the first frost, the decline in content is first rapid, amounting to 51% in the first month and a half, then a further loss of 20% by the end of 5½ months. The vitamin C content of tomatoes and carrots also exhibits a similar seasonal rise and decline.

In grapefruit and oranges, the content shows a monthly variation, which is dependent on many factors, such as gas ripening, transportation, and variable lengths of storage.

TABLE VI

SEASONAL VARIATION OF VITAMIN C CONTENT OF RAW POTATOES

Dates of samplings	Condition	Average vitamin C content	
		Mg./100 gm. wet wt.	Mg./100 gm. dry wt.
Aug. 23, 1940	Flowering plant	18.4	64.5
Aug. 28 and 30	Not flowering	20.8	75.4
Sept. 3 and 6	Not flowering	27.9	101.8
Sept. 10 and 13	Frost, Sept. 10	34.6	124.4
Sept. 18 and 20	Frost, Sept. 20	27.4	102.5
Sept. 24 and 27		23.0	82.2
Oct. 1 and 4	Dug up and stored Sept. 30	23.5	93.7
Oct. 8 and 12		21.6	91.4
Oct. 15 and 18		21.2	87.2
Oct. 22 and 25		17.8	71.9
Oct. 29 and Nov. 1	Stored one month	17.0	70.0
Nov. 5 and 8		15.0	54.6
Nov. 12 and 15		13.9	51.3
Nov. 19 and 22		13.3	52.8
Nov. 26 and 29		12.2	47.3
Dec. 3 and 6	Stored two months	14.5	53.7
Dec. 10 and 13		12.1	43.6
Dec. 17 and 20		12.5	45.7
Dec. 24		13.8	49.6
Jan. 7 and 10, 1941	Stored three months, six days	10.4	37.2
Jan. 14 and 17		9.0	32.4
Jan. 21 and 24		8.6	31.9
Jan. 28 and 31		11.4	42.8
Feb. 4 and 7	Stored four months	8.0	30.5
Feb. 11 and 14		9.8	36.6
Feb. 21		7.0	30.2
Feb. 25 and 28	Stored five months	9.9	37.2

Comparison of Institutionally and Home-cooked Foods

Where possible, the values obtained during the Athabasca Hall survey were compared with those obtained with locally grown vegetables and fruits. In general the tendency was for the vitamin C content of the foods cooked in an institution to be less than the content of the home-cooked foods.

Comment

This paper presents a general survey of fruits and vegetables that form sources of vitamin C in Alberta diets. Though the problem is local in nature, the results obtained are in all probability applicable to all Western Canada. The importance of the work lies in the furnishing of actual values of the vitamin C content of Alberta grown vegetables and fruits, and the supplementing of these data by those obtained during the investigation of an existing dietary.

Acknowledgments

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ENZYME INHIBITION BY DERIVATIVES OF PHENOTHIAZINE

III. CATALASE, CYTOCHROME OXIDASE, AND DEHYDROGENASES[1]

By H. Bruce Collier[2] and Della E. Allen[3]

Abstract

The inhibition of liver catalase and of cytochrome oxidase by leucophenothiazone has been confirmed by manometric methods. Phenothiazine sulphoxide is a powerful catalase inhibitor, its activity being much greater at pH 5.3 than at neutrality.

The succinoxidase activity of beef heart is inhibited by phenothiazone and by thionol. Phenothiazone in its oxidized form acts upon the succinic dehydrogenase, while the leuco form inhibits the cytochrome oxidase.

Phenothiazone is reduced by the yeast lactic dehydrogenase system, and the enzyme activity is markedly decreased. Urease is also partially inhibited, whereas the effect of phenothiazone upon *d*-amino-acid oxidase is almost negligible.

The possible relationship of these findings to the action of phenothiazine upon living organisms is discussed.

Introduction

The first paper in this series (5) described the inhibition of catalase and of cytochrome oxidase by leucophenothiazone, leucothionol, and thionol, all of which are oxidation products of phenothiazine in the animal body (4). This work marked the beginning of a general program designed to explain the anthelmintic activity of the drug phenothiazine. The second paper in the series (7) described the inhibition of serum cholinesterase by phenothiazone and by certain other derivatives of phenothiazine.

The present paper is, in part, an extension of the previous work on catalase and cytochrome oxidase, employing a more precise manometric technique. It also deals with the inhibition of succinic dehydrogenase, as was reported in a preliminary note (6), and records a similar effect upon lactic dehydrogenase; *d*-amino-acid oxidase, on the other hand, was only slightly inhibited.

Experimental

A. Inhibition of Catalase

Methods

Phenothiazone and phenothiazine sulphoxide were prepared by the methods of Pummerer and Gassner (17); the former was recrystallized from water, giving brick-red crystals melting at 165° C. (corr.); the sulphoxide was recrystallized from acetone. Leucophenothiazone was prepared by reduction of phenothiazone by Bernthsen's method (3).

[1] *Manuscript received in original form September 18, 1942, and as revised November 5, 1942. Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada.*

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[3] *Research Assistant.*

A catalase extract was prepared from guinea-pig liver, as previously described (5). This extract was diluted 1 : 100 for use, and its activity was estimated manometrically in a Summerson differential manometer, at 15° C. The enzyme was made up to 1.0 ml. in buffer, $M/15$ in each of acetate and phosphate, pH 6.8; this was mixed with 0.3 ml. of 3% hydrogen peroxide at zero time. The control vessel contained substrate and buffer but no enzyme; under these conditions, the amount of decomposition in five minutes was virtually proportional to enzyme concentration. Inhibitors were dissolved in the buffer in each vessel, and were in contact with the enzyme during the 15 min. equilibration period.

Effect of Leucophenothiazone

This derivative was added as an alcoholic solution; the same volume of alcohol (never more than 0.1 ml.) was added to the standard enzyme, as it definitely stimulated catalase activity. The degree of inhibition thus observed is recorded in Table I; 50% inhibition requires about 6×10^{-6} M concentration of leucophenothiazone.

TABLE I

INHIBITION OF CATALASE BY LEUCOPHENOTHIAZONE

0.0003 ml. enzyme in 1.3 ml. total; pH 6.8, 15° C.

Inhibitor concentration, $M \times 10^6$	Oxygen liberated in five min., μl.	Inhibition, %
0	181	0
4	100	45
19	62	66
38	42	77

Effect of Phenothiazone

It was thought that the semiquinone form of phenothiazone might be the actual inhibitor. To test this hypothesis, phenothiazone was heated in glacial acetic acid or in 7 N hydrochloric acid, which converts a large proportion into the free radical form (see Granick, Michaelis, and Schubert (9)). This acid solution was then added to neutral acetate–phosphate buffer to give a resultant pH value of 5.3. No inhibition was observed, however, when catalase was added, and as previously noted (5) phenothiazone had no effect upon the enzyme at pH 7.

Effect of Sulphoxide

Phenothiazine sulphoxide was found to be a powerful inhibitor of catalase. For testing at pH 6.8 the compound was added in alcoholic solution, as in the case of leucophenothiazone. Table II gives the results of these tests, where it is seen that the point of 50% inhibition is at about 2.5×10^{-5} M concentration.

TABLE II

INHIBITION OF CATALASE BY SULPHOXIDE AT pH 6.8

0.0003 ml. enzyme in 1.3 ml. total volume; 15° C.

Sulphoxide concentration, $M \times 10^6$	Oxygen liberated in five min., μl.	Inhibition, %
0	232	0
0.8	197	15
7.7	156	33
26	113	51
77	97	58
230	44	81

The effect of increasing acidity upon the inhibition was also tested, in the hope of throwing light upon the possible role of the semiquinone form. The sulphoxide was dissolved by heating in 1 *N* hydrochloric acid, which gives the sulphonium chloride, according to Barnett and Smiles (1). This solution has a high concentration of the semiquinone of phenothiazine, as described by Michaelis, Granick, and Schubert (16). It was made up to contain varying concentrations of the sulphoxide and was added to the neutral acetate–phosphate buffer in sufficient volume to give a final pH value of 5.3. The inhibition of catalase, using these solutions, is shown in Table III; about 1×10^{-6} *M* concentration of sulphoxide is required to produce 50% inhibition.

TABLE III

INHIBITION OF CATALASE BY SULPHOXIDE AT pH 5.3

0.0010 ml. enzyme in 1.3 ml. total volume; 15° C.

Sulphoxide concentration, $M \times 10^7$	Oxygen liberated in five min., μl.	Inhibition, %
0	210	0
3	128	39
30	79	62
300	39	81

B. INHIBITION OF HEART MUSCLE ENZYMES

Methods

The preparation of phenothiazone and of its leuco form have already been described. Thionol was prepared by the original method of Bernthsen (3); the crude product was dissolved in sodium hydroxide and was precipitated by the addition of hydrochloric acid, giving an amorphous material melting at 134 to 136° C. (uncorr.). As noted by Granick, Michaelis, and Schubert (9), there is no satisfactory method for preparing pure thionol.

The enzyme was prepared from beef heart by the methods of Stotz and Hastings (18) and of Keilin and Hartree (12). Cytochrome-*c* was isolated from beef heart by the method of Keilin and Hartree (11).* Both types of enzyme preparation gave similar results, as far as the inhibitions were concerned. Cytochrome-*c*, added to 3×10^{-5} *M* concentration, increased the activity of the Keilin-Hartree enzyme, but did not affect that of the Stotz-Hastings preparation; nor did it affect the inhibitions observed. The Stotz-Hastings enzyme was used throughout in the experiments to be described—0.5 ml. of suspension in a total volume of 2.5 ml.

All measurements of enzyme activity were made manometrically, at 37° C. and at pH 7.3 in *M*/15 phosphate buffer; the control vessels contained enzyme but no substrate. Inhibitors were dissolved in the buffer, and were in contact with the enzyme during the 15 min. equilibration period, before mixing with substrate. Leucophenothiazone was most conveniently added by dissolving phenothiazone in the buffer, then reducing it with a little ascorbic acid. The same amount of ascorbic acid when added to the standard enzyme had no noticeable effect.

Succinoxidase Activity

The oxygen uptake of the total heart preparation was measured in the presence of 0.02 *M* succinate. Table IV indicates that the succinoxidase activity was slightly stimulated by low concentrations of phenothiazone and was strongly inhibited by higher concentrations. About 1.2×10^{-4} *M* phenothiazone gives a 50% decrease in oxygen consumption.

TABLE IV

INHIBITION OF HEART SUCCINOXIDASE BY PHENOTHIAZONE

0.5 ml. enzyme in 2.5 ml. total; pH 7.3, 37° C.

Phenothiazone concentration, $M \times 10^5$	Oxygen uptake in 60 min., μl.	Change in oxygen uptake, %
0	162	0
4	180	+ 11
8	107	− 34
16	61	− 62
50	0	−100

Thionol showed a much smaller inhibitory power. Under conditions similar to the above, the following results were obtained (oxygen consumption in 60 min.):—

Normal enzyme	128 μl.
Thionol, 0.001 *M*	101 μl.
Thionol, 0.003 *M*	32 μl.

* *In these preparations the Waring Blendor (Waring Corpn., 1697 Broadway, New York City) was found very useful for mincing tissue, to replace more tedious methods of grinding.*

Dehydrogenase Activity

The dehydrogenase activity of the heart preparation was measured in the presence of 0.02 *M* succinate, 0.002 *M* potassium cyanide, and 0.0004 *M* methylene blue. As seen in Table V, phenothiazone and thionol were strongly inhibitory, while leucophenothiazone had virtually no effect.

TABLE V

INHIBITION OF SUCCINIC DEHYDROGENASE

0.5 ml. enzyme in 2.5 ml. total; pH 7.3, 37° C.

Inhibitor	Concentration, *M*	O_2 uptake in 60 min., μl.		Decrease in O_2 uptake, %
		Normal	Treated	
Phenothiazone	0.0005	125	54	57
Leucophenothiazone	0.0005	110	96	13
Thionol	0.001	122	46	62

Cytochrome Oxidase Activity

The oxidase activity was determined in the presence of 0.01 *M p*-phenylenediamine. The results in Table VI indicate that phenothiazone had no effect; leucophenothiazone and thionol showed definite inhibitions.

TABLE VI

INHIBITION OF CYTOCHROME OXIDASE

0.5 ml. enzyme in 2.5 ml. total; pH 7.3, 37° C.

Inhibitor	Concentration, *M*	O_2 uptake in 30 min., μl.		Decrease in O_2 uptake, %
		Normal	Treated	
Phenothiazone	0.0005	176	178	0
Leucophenothiazone	0.00016	208	119	43
Thionol	0.003	248	194	22

C. OTHER ENZYME SYSTEMS

Inhibition of Lactic Dehydrogenase

A few preliminary experiments have been done with lactic dehydrogenase, prepared from yeast by treatment with acetone by Bernheim's method (2). A phosphate extract of the dried powder was tested on 0.2 *M dl*-lactate as substrate, in the presence of 5×10^{-4} *M* methylene blue, 0.2 *M* hydroxylamine, and a trace of boiled yeast extract. Phenothiazone, added to a concentration of 0.0005 *M*, was completely reduced to the leuco form. The results of a typical experiment were as follows (oxygen consumption in 60 min.):—

1.0 ml. yeast extract in 2.5 ml. volume	242 μl.
In presence of leucophenothiazone	137 μl.

Effect on d-Amino-acid Oxidase

This enzyme was prepared from pig kidney cortex by treatment with acetone according to Krebs (14). It was tested in phosphate buffer in the presence of 0.04 *M dl*-alanine. The activity was only slightly reduced by phenothiazone at 0.0005 *M* concentration, the normal oxygen consumption of 135 µl. in one hour being decreased to 110 µl. Leucophenothiazone in the same concentration had a negligible effect.

Effect on Urease

Because of the possibility of oxidation of free sulphydryl groups by phenothiazone, its effect upon urease was tested. Arlcourease was added to 2.5% urea in *M*/15 phosphate at pH 7.0, and the rate of decomposition was followed, at 37° C., by estimating the ammonia. The ammonia formed, as milliequivalents per millilitre per hour, was as follows:—

No inhibitor	0.065 m.e.
Phenothiazone, 12×10^{-5} *M*	0.054 m.e.
Phenothiazone, 50×10^{-5} *M*	0.035 m.e.

It is therefore apparent that the dye, under these conditions, produces a partial inhibition of the enzyme activity.

Discussion

The inhibition of the enzymes catalase and cytochrome oxidase by leucophenothiazone has been confirmed by manometric methods. As previously suggested (5), this inhibition is attributed to the phenolic OH group, which probably forms covalent complexes with the active iron atoms of these haemin catalysts. (In the previous paper it was erroneously suggested that leucophenothiazone acts by combining with cytochrome, rather than with the oxidase, thus preventing reoxidation of the cytochrome.)

Phenothiazine sulphoxide has also been found to be a very powerful catalase inhibitor. Its activity at pH 5.3 is much greater than at pH 6.8, which suggests that the sulphonium ion is the active agent. Michaelis and his co-workers (9, 16) have discussed the strong resonance of the thiazine molecule, and this may be related to the enzyme inhibitions, but at present there is no evidence in favour of such a speculation.

In the case of the beef heart preparation it has been found that phenothiazone inhibits the succinoxidase activity (aerobic oxidation of succinate). On further analysis it was shown that the oxidized form of phenothiazone inhibits the dehydrogenase factor (i.e., that which is active in the presence of cyanide and methylene blue), while the leuco form of the dye inhibits the cytochrome oxidase activity.

Stotz and Hastings (18) correlated the inhibition of succinoxidase by dyes with the oxidation–reduction potentials of the dyes. Our results with phenothiazone correspond very closely with their findings. Thus, phenothiazone has an E_0' value of + 0.127 volts at pH 7.3, as interpolated from the data of

Granick, Michaelis, and Schubert (9)*; the degree of inhibition is similar to that in the presence of naphthosulphonate indophenol, with an E_0' value of + 0.105 volts. This suggests that phenothiazone acts upon the dehydrogenase by virtue of its relatively high oxidation–reduction potential, which is higher than that of the succinate–fumarate system or of cytochrome-*b*. The findings with urease suggest that the dye may have some effect upon the free SH groups of this enzyme, although it cannot be regarded as a very active oxidant for these groups.

Weil-Malherbe (19) and Keilin and Hartree (13) have observed an inhibition of heart succinoxidase by pyocyanin, which led to our experiments with phenothiazone. Keilin and Hartree proposed that pyocyanin may attack an intermediate link between the dehydrogenase and cytochrome oxidase; this may also be true of phenothiazone, where the over-all oxygen uptake is more strongly inhibited than is the dehydrogenase activity alone.

It is impossible at present to correlate these enzyme inhibitions, observed on mammalian tissue *in vitro*, with the anthelmintic, insecticidal, and bactericidal actions of the phenothiazine derivatives. It will be necessary to examine the effect of the compounds upon the enzyme systems of the actual organisms. Nevertheless, it may be of some significance that McIlwain (15) has proposed that anthelmintic drugs act as narcotics, and the observed inhibition of succinic and of lactic dehydrogenases fits in with Quastel's (10) well known theory of narcotic action.

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* *For thionol, E_0' is + 0.142 volts at pH 7.3, calculated from the data of DeEds and Eddy* (8).

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ERRATUM

Page 246, line 1 of footnote 1, for "August 28" read "August 3".

CANADIAN JOURNAL OF RESEARCH

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(ii) *Photographs:*—Prints should be made on glossy paper, with strong contrasts; they should be trimmed to remove all extraneous material so that essential features only are shown. Photographs should be submitted *in duplicate;* if they are to be reproduced in groups, one set should be so arranged and mounted on cardboard with rubber cement; the duplicate set should be unmounted.

(iii) *General:*—The author's name, title of paper, and figure number should be written on the back of each illustration. Captions should not be written on the illustrations, but typed on a separate page of the manuscript. All figures (including each figure of the plates) should be numbered consecutively from 1 up (arabic numerals). Reference to each figure should be made in the text.

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